47 INTRAPERITONEALLY TRANSPLANTED FETAL RAT INTESTINE: MORPHOLOGY AND FUNCTION

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In previous studies we have demonstrated that transplantation of fetal rat intestine into the peritoneal cavity of adult rats is possible and that the transplanted gut undergoes differentiation and maturation. Histology of these transplants showed normal appearance. In a further study we wanted to examine the functional integrity of the transplanted intestine after anastomosis to the intestine of the host. Disaccharidase activities were measured in the transplant and compared to the "control" activities found in the intestine of the host rat. Maltase and sucrase activities of the transplant were approximately 60% of control, lactase activity was the same for transplant and control. The capacities of glucose and amino acid uptake of the transplanted epithelium were not different from control. Peristalsis of the transplant was measured by electromyographic recordings: slow wave frequency and spike activity were at the same levels as in controls. These results demonstrate that the transplanted fetal rat intestine has the structural and functional characteristics of the normal rat small bowel and should be considered as an alternative to the transplantation of adult bowel.

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48 ANP INDUCES WATER AND ELECTROLYTE SECRETION IN THE IN VIVO RAT PERFUSED JEJUNUM.

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Atrial natriuretic peptide (ANP) is involved in the renal regulation of water and electrolyte homeostasis. ANP exerts its effects through the stimulation of the guanylate cyclase (GC)-cGMP system. Conflicting data have been reported concerning the effects and the mechanism of action of ANP in the intestine. Aims of the work were: 1) to see whether ANP affects the intestinal water and electrolyte transport and 2) to investigate its mechanism of action and namely the binding of ANP to intestinal cells or membranes and its effects on the GC-cGMP system. Intravenous administration of ANP (0.3ug/Kg/30min) resulted in a significant shift of water, sodium and chloride absorption toward secretion in the vivo perfused rat jejunum. Such effect was prompt, but of short duration (less than 10 minutes on a continued ANP infusion). No effect was observed on potassium transport. The effects of the addition of 3-28 rat ANP in a concentration range of 10^{-8} to 10^{-5}M on GC and on cGMP were studied using rat jejunal homogenates and rat jejunal cells. GC and cGMP were measured at various times after the addition of ANP. 2 minutes after the addition on ANP, a modest (1.5 times) increase of both GC and cGMP was observed which rapidly returned to basal levels. No specific binding was obtained by incubating ^{125}I-ANP with basolateral membranes, cells or homogenates prepared from rat jejunum. We conclude that ANP may play a role as a short-lived modulator in the intestinal regulation of water and electrolyte homeostasis. The mechanism of such interaction appears to be indirect, since it does not involve the GC-cGMP system nor is mediated by specific binding sites on the enterocyte.

49 E.COLI HEAT-STABLE TOXIN PRODUCTION MAY BE ACQUIRED THROUGH CONJUGATION AND MODIFIED BY BACTERIA OTHER THAN E.COLI

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We have previously described two E.Coli heat stable (ST)-like toxins. The first, produced Citrobacter freundii (Cf), has the same 18 amino acid sequence of E. Coli STIa. 1) The second, produced by Klebsiella pneumoniae (Kp) is similar, but not identical to E.Coli STIa and to Cf ST in that it does not react with monoclonal antibodies raised against pure E.Coli STIa. 2) To see whether transfer of toxigenic ability may occurr between different bacterial species we have performed conjugal bacterial mating experiments. We used ST positive Cf (strain AG55) as the donor and the following ST negative recipients: 1) E.Coli, 2) Kp P89 and 3) Kp strain AL55- (which had spontaneously lost the ability to elaborate the ST-like toxin). Mixed cultures were incubated for 2 hours at 37 C and then plated onto XLD agar containing an antibiotic to which only Cf was sensitive. Grown colonies were individually tested for ST production by an ELISA test with monoclonal antibodies and by the classic suckling mouse assay (SMA). Results: both E.Coli and KpP89 acquired the ability to produce the toxin, as indicated by the positivity of both SMA and ELISA. The estimated transfer frequency for E.Coli was $9x10^{-2}$ matings. KpAL55- showed positivity in the SMA but not in the ELISA, thus showing that it had acquired the ability to elaborate a toxin somehow different from that of the donor. To assess whether the acquired toxigenic ability was itself transmissible, the new ST+E.Coli was incubated with an ST-Cf. The latter became ST+.

In conclusion: 1) Different enterobacteria may trade ST plasmids by conjugation; 2) the ability to produce ST is acquired together with the ability to infect other bacteria; 3) selected Klebsiella strains acquire the capability of producing ST, which is successively modified through a post-conjungative, yet unknown, mechanism.

1) Guarino A et al. J Clin Microbiol 25:110-114,1987

2) Guarino A et al. Ped Res 25:514-518, 1989

50 ADAPTIVE RESPONSE OF ILEAL MUCOSA TO MALNUTRITION IN THE RAT: ROLE OF POLYAMINES.

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Mucosal disaccharidases and ornithine decarboxylase (ODC) activities were measured in malnourished (MN), preweaning (19 days), post weaning (24 d) and young adult (37 d) rats. Malnutrition resulted in decreased body weight, intestinal weight, DNA and protein content. Mucosal Prot/DNA ratios were elevated in the ileal segments of 24 and 37 d rats. Mucosal sucrase(s) and (ODC) specific activities and polyamine content were significantly elevated in the ileal segment of 24 and 37 day old MN rats only. Results for 37 d old rats were: Sucrase IU/gPr 51.2±7.7 (C) versus 88.8±12.4 (MN), p<0.01 ODC pmol/h/gPr 11.4±6.2 (C) versus 46.3±2.1 (MN) p<0.01, Putrescine nmole/10cm mucosa 1.6±0.7 (C) versus 6.1±4.3 (MN) p<0.05. These increased specific activities were located in the mature enterocytes at the villous tip. DFMO administration for 4 days inhibited mucosal ODC and completely abolished the adaptive rise of ileal sucrase activity. Sucrase in DFMO treated MN rats = 28.7±9.0 IU/gPr. We conclude that the intestinal response to reduced food intake is age related and differs in the jejunum and ileum: ODC and polyamines are involved in ileal adaption to malnutrition in post weaned and adult rats.

51 PHENOTYPIC AND GENOTYPIC VARIATION IN GIARDIA LAMBLIA

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There is no simple explanation for the varied spectrum of clinical disease produced by Giardia. This may partly relate to strain variation but as yet no virulence factors have been identified. We have characterised 12 human Giardia isolates from UK, USA and travellers to Asia and 2 isolates from animals by (1) [^{35}S]-methionine radiolabelling profiles, (2) isoenzyme analysis, (3) heat-shock proteins and (4) DNA analysis by RFLP. [^{35}S]-methionine profiles resolved by SDS-PAGE enabled isolates to be separated into 5 groups, isoenzyme analysis into three groups according to malic enzyme isoenzymes (but not G6PD or hexokinase) and RFLP analysis with Hind III and a cloned Giardia DNA probe revealed polymorphisms in 3 isolates, 2 of which also exhibited the malic enzyme zymodeme differences. Heat shock proteins (94,81,70,30kDa) were demonstrated in all Giardia isolates but did not discriminate between them. We have used these approaches to study Giardia isolate variation in a patient with immunodeficiency and chronic giardiasis and have shown that during several unsuccessful treatments with metronidazole both the phenotypic ([^{35}S]-methionine profile, isoenzymes) and genotypic (RFLP) characteristics of the isolate changed, probably indicating selection of a resistant clone since sensitivity to metronidazole decreased >40-fold during the 3 month study. Thus, these techniques can identify phenotypic and genotypic differences between Giardia isolates and may prove useful typing systems for identification of virulence factors.

52 TOBEC DISCRIMINATES BETWEEN EXTRA- AND INTRACELLULAR BODY FLUIDS

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Measurement of total body electrical conductivity (TOBEC) a new non-invasive method for the determination of fat-free mass, operates on the principle that body fluids perturb an electromagnetic field proportional to their conductivity,which is dependent on the amount of the different cations and anions present. We evaluated whether differences between the conductivities of intracellular- (ICF) and extracellular body fluids (ECF) can be measured with the TOBEC instrument (EM-Scan,HP-2). Solutions were made up to approximate the concentrations of ions that are present in ICF and ECF. Phantoms (flat plastic bags) containing solutions with ICF:ECF (%) ratios of a) 30:70 (premature infant); b) 39:61 (term newborn infant) and c) 44:56 (infant, 6months of age) were made for each of the volumes 0.5,1,2 and 3 liters. Three regression lines were calculated from the TOBEC readings obtained from the phantoms con - taining solutions a,b or c.Significant differences between the three regression lines indicated that even small differences in ICF:ECF ratios at volumes between 0.5 - 3 liters can be measured with the TOBEC instrument. Our findings further indicate that the fat-free mass of premature infants derived from TOBEC measurements will be systematically overestimated unless age specific ICF:ECF ratios are introduced into the prediction equations.